

SOLANOCARDINOL: A STEROIDAL ALKALOID FROM *SOLANUM NEOCARDENASII*

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Key Word Index—*Solanum neocardenasii*; Solanaceae; potatoes; glycoalkaloid; spectroscopic analyses.

Abstract—The structure of a new steroidal alkaloid from *Solanum neocardenasii*, solanocardinol, has been characterized by mass spectrometry, 2D-NMR and chemical methods. In contrast to the closely related spirofuranopiperidine the configuration of this compound at C-16 is β . The relevance of solanocardinol to the chemotaxonomy of *Solanum* species is discussed.

INTRODUCTION

The aglycones of *Solanum* glycoalkaloids are products of a common biosynthetic pathway that diverge in the cyclization of the cholesterol side chain. Thus, the spiro-solanes and solanidanes which are the common alkaline ring systems of tuber-bearing *Solanum* species that are genetically closely related to *S. tuberosum*, have been postulated to be derived from a common intermediate [1] (Fig. 1) in which the OH-16 is β . The alkalines of *S. aculeatum* (which is not tuber-bearing and not closely related to *S. tuberosum*), 3-desamino-3 β -hydroxysolanocapsine (**1a**) [2] and aculeamine (**1b**) [3], on the other hand, are products of the cyclization of an α OH-16 precursor.

We now describe the isolation and characterization of an alkaline from the wild tuber-bearing [4], pest resistant [5] species, *S. neocardenasii*, that we have named solanocardinol and which is isomeric to 3-desamino-3 β -hydroxysolanocapsine, but is sterically consistent with the steroidal alkaloid biosynthetic pathway of tuber-bearing *Solanum* species. A detailed analysis of the NMR data is presented as there are significant differences in

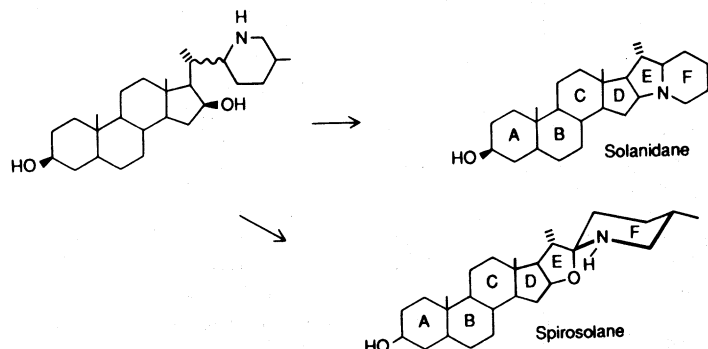
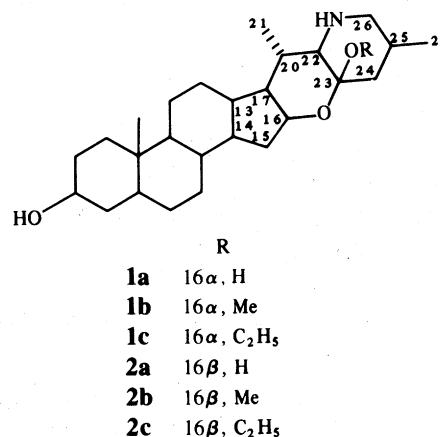


Fig. 1. Biosynthetic pathway for formation of ring E of *Solanum* steroidal alkaloids.

both the ^{13}C and ^1H NMR spectra of solanocardinol and 3-desamino-3 β -hydroxysolanocapsine.

RESULTS AND DISCUSSION

The mass spectra of the alkalines isolated from aqueous, methanolic and ethanolic acid hydrolysis of the glycoalkaloid fraction of *S. neocardenasii* were identical, within experimental parameters, to the mass spectra of 3-desamino-3 β -hydroxysolanocapsine [2], aculeamine [3] and the 23-ethoxyderivative (**1c**) [3], respectively. The prominent ion in the spectra of the methoxy and ethoxy compounds arising from retro-Diels–Alder fragmentation (Fig. 2) appears to be diagnostic for the E/F ring structure of these compounds.

NMR spectra were obtained on the ethoxy compound (**2c**) in order to eliminate the epimeric equilibration that may occur at C-23 in **2a**. The shift assignments for the carbons of rings D, E and F are given in Table 1. The supporting DEPT, ^1H 2D-J resolved, heteronuclear COSY and homonuclear COSY data are also presented. The ^{13}C NMR assignments for C-20 to C-24 of 22,26-epimino-23 β -ethoxy-16 α ,23-epoxy-5 α ,22 α ,25-cholestane-3 β -ol [3] are significantly different from our assignments for the same carbons in ethoxysolanocardinol (shift as-

signments for the carbons in rings A, B and C of the two compounds are within 0.2 ppm). For the most part, these shift differences may reflect the difference in configuration at C-16; for example, the signal for C-16 in ethoxysolanocardinol is 5.1 ppm upfield from that observed for the ethoxy compound isolated for *S. aculeatum*. This is consistent with the ethoxy substituent β in the former compound and α in the latter [6]. The β -oxo configuration would be expected on biogenetic grounds because the major spirosolane alkaloids of the tuber-bearing *Solanum* species closely related to *S. neocardenasii* (including tomatidine which is the other alkaloid found in this species) also have this configuration. However, the large difference for the shifts assigned to C-22 cannot be readily explained. Our assignment for C-22 (59.1) is appreciably upfield from the C-22 resonance in the ethoxy compound isolated from *S. aculeatum* (68.7). However, our assignment is supported by the data obtained in the other NMR experiments (see Table 1). Besides the experimental data, no other shift assignment seems possible because the signal at δ 68.8 can be unambiguously assigned to C-16. The difference in assignment for C-24 is intriguing; our assignment for this carbon is similar to that reported for the C-24 in the methoxy compound (37.9). Carbons 20, 22 and 25 may be assumed to have the same configuration as found in tomatidine on biosyn-

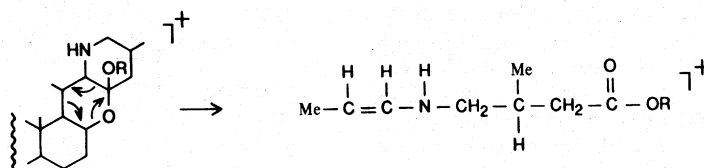


Fig. 2. Retro-Diels–Alder fragmentation of alkylated solanocardinols.

Table 1. ^{13}C NMR spectral data and corroborative NMR data for E and F ring carbons of ethoxysolanocardinol

C	$\delta\text{C}_{\text{obs}}$	δC (ref. [3])	NAP*	Heteronuclear COSY δH	Homonuclear COSY		
					δH_A	δH_B	δH_C
14	53.5	54.8	1	NCP	—	—	—
15	29.7	28.2	2	1.22	4.20 (H_{16})	—	—
16	68.8	73.9	1	4.20	1.22 (H_{15})	1.05 (H_{17})	—
17	58.3	60.3	1	1.05	2.10 (H_{20})	4.20 (H_{16})	—
18	14.7	13.6	3	0.75	—	—	—
19	12.4	12.3	3	0.75	—	—	—
20	33.7	32.9	1	2.10	2.55 (H_{22})	1.05 (H_{17})	0.08 (H_{21})
21	18.7	15.4	3	0.80	2.10 (H_{20})	—	—
22	59.1	68.7	1	2.55	2.10 (H_{20})	—	—
23	97.1	98.4	0	—	—	—	—
24	37.4	40.2	2	1.70	—	—	—
25	28.2	30.8	1	2.00	0.80 (H_{27})	3.0 (H_{26})	—
26	54.4	54.0	2	3.00	2.20 (H_{25})	—	—
27	18.7	18.6	3	1.1	2.20 (H_{25})	—	—
28	54.8	54.3	2	3.35, 3.4	1.15 (H_{29})	—	—
29	15.4	15.4	3	1.15	3.35, 3.4 (H_{28})	—	—

*Number of attached protons as determined by ^1H 2D-J resolved and DEPT spectra.

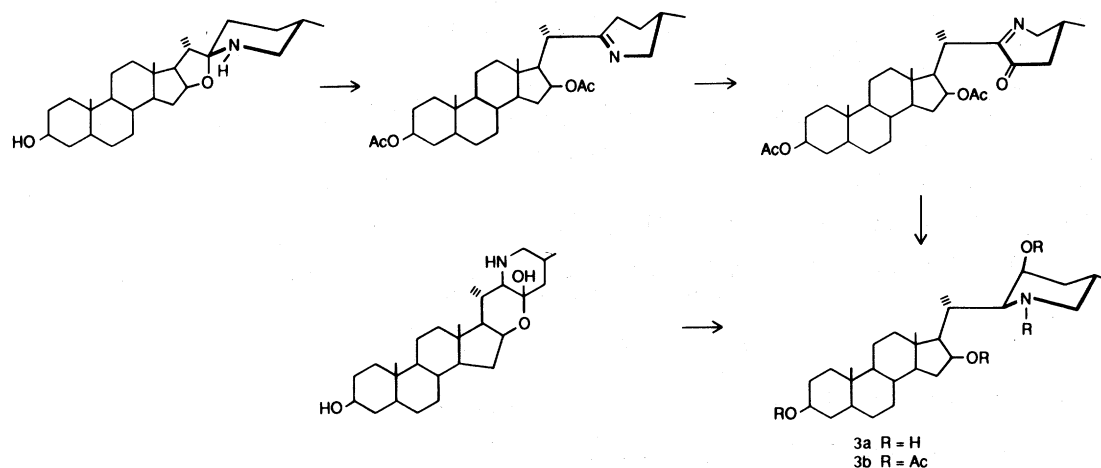


Fig. 3. Synthesis of 3 β ,16 β ,23-trihydroxy-22,26-epimino-5 α -cholestane from tomatidine (a) and solanocardinol (b).

thetic grounds, however, these have not been experimentally established.

The inconsistency of the NMR data prompted us to confirm the structure of solanocardinol by chemical means. Sodium borohydride reduction of solanocardinol yielded a pair of C-23 isomers of the trihydroxy compound 3a. The acetylated derivatives (3b) were identical by mass spectrometry to the compounds having the same R_f (on TLC) obtained from the degradation of tomatidine (Fig. 3) [7].

The carbohydrate portion of the glycoalkaloid from which solanocardinol was obtained was identified as lycotetraose (*O*- β -D-glucopyranosyl-(1 \rightarrow 2_{Glc})-*O*- β -D-xylopyranosyl-(1 \rightarrow 3_{Glc})-*O*- β -D-glucopyranosyl-(1 \rightarrow 4_{Gal})- β -D-galactopyranose) by monosaccharide analysis and by comparison of the carbohydrate region of the ^1H and ^{13}C NMR spectra with tomatine.

EXPERIMENTAL

Solanum neocardenasii (PI 498129, PI 502642) collected in Bolivia was obtained from the Potato Introduction Station, Sturgeon Bay, WI, and grown in the field. Foliage was collected, immediately frozen in liquid N_2 , and subsequently lyophilized. NMR data were obtained at 100 MHz for ^{13}C and 400 MHz for ^1H spectra. Samples were dissolved in CDCl_3 with TMS as int. standard. MS were obtained by GC-MS in the direct probe mode.

Isolation of solanocardinol and oxo derivatives. *Solanum neocardenasii* dry leaf powder (90 g) was extracted with a soln of 800 ml MeOH and 100 ml 50% aq. HOAc. The residue was extracted with another 100 ml of solvent, the solvent extracts combined, concd to 500 ml and made basic with conc. NH_4OH . After gentle heating, the ppt. that formed was filtered and washed with 10% NH_4OH . The ppt. which contained ca 90% of the unknown glycoalkaloid (the other 10% being tomatidine) was hydrolysed in either 5% HCl -MeOH, 5% HCl -EtOH or 0.5 M H_2SO_4 . Alkalines were purified by CC and prep. HPLC. *Solanocardinol*. MS (probe) 70 eV m/z (rel. int.): 431 $[\text{M}]^+$ (50), 413 $[\text{M}-18]^+$ (4), 161 (7), 157 (22), 156 (11), 142 (100), 130 (29), 125 (40), 112 (13), 84 (13), 70 (53). *Methoxysolanocardinol*. MS (probe) 70 eV m/z (rel. int.): 445 $[\text{M}]^+$ (3), 430 $[\text{M}-15]^+$ (5), 415

$[\text{M}-30]^+$ (5), 414 $[\text{M}-31]^+$ (4), 413 $[\text{M}-32]^+$ (3), 273 (3), 229 (4), 144 (3), 171 (20), 112 (10), 84 (5), 70 (100). *Ethoxysolanocardinol*. MS (probe) 70 eV m/z (rel. int.): 459 $[\text{M}]^+$ (1), 444 $[\text{M}-15]^+$ (1), 430 $[\text{M}-29]^+$ (2), 413 (2), 273 (4), 185 (13), 184 (4), 112 (15), 84 (15), 70 (100), 57 (65), 55 (59)).

(22S,25S) 22,26-Acetylepimino-5 α -cholestan-3 β ,16 β ,23-triacetate. NaBH_4 (10 mg) was added to a soln of 5 mg of solanocardinol in 1 ml of 95% EtOH and stirred overnight at room temp. The soln was then dild with 2 ml H_2O and extracted with CHCl_3 (2×2 ml). The CHCl_3 washed with H_2O , dried (MgSO_4) and concd under N_2 . The product, a mixt. of 23 α and 23 β isomers, was crystallized from MeOH- H_2O and acetylated with Ac_2O -pyridine (70 $^\circ$, 2 hr) and the 22R isomers isolated by prep. TLC on silica gel using EtOAc-cyclohexane (1:1). MS (probe) 70 eV m/z (rel. int.): 541 (12), 466 (12), 438 (15), 198 (100), 156 (88), 114 (22).

(22RS,25S) 22,26-Acetylepimino-5 α -cholestan-3 β ,16 β ,23-triacetate. The procedure for the degradation of tomatidine described in ref. [7] was used with minor modifications. Compounds were purified by silica gel CC or TLC using CHCl_3 -MeOH (20:1). The compounds were completely acetylated as described above. Isolation of the mixt. of acetylated 22R and S isomers was also achieved as described above. The MS was identical to that obtained for the isomers derived from reduction of solanocardinol.

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EBURNAMINOL AND LARUTENSINE, ALKALOIDS FROM *KOPSIA LARUTENSIS*

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Key Word Index—*Kopsia larutensis*; Apocynaceae; indole alkaloid; eburnane; structural elucidation.

Abstract—Bark and stems of *Kopsia larutensis* were extracted to afford six alkaloids two of which are new, viz. eburnaminol and larutensine. Both possess the eburnane skeleton and the latter has an additional ether linkage between C-16 and C-18. The structures of the new alkaloids were elucidated by spectral methods.

INTRODUCTION

The genus *Kopsia* (subfamily Plumerioideae, tribe Rauvolfiae) incorporates ca 30 species in China, India, Thailand, Philippines, Indonesia and Malaysia. Several Malaysian *Kopsia* have been studied for their alkaloid contents: *K. deverrei* L. Allorge & L. E. Teo [1, 2], *K. pauciflora* Hook f. [3], *K. singapurensis* Ridl. [4–6], *K. fruticosa* A. DC. [7], *K. profunda* Markgraf [8] and *K. dasyrachis* Ridl. [9, 10]. We now present our work under the scientific cooperation programme between University of Malaya and Centre National de la Recherche Scientifique [11] regarding Malaysian *K. larutensis* King & Gamble which is a tree ca 2 m high with white flowers.

RESULTS AND DISCUSSION

Stems and barks of *K. larutensis* were extracted with dichloromethane and conventional methods of alkaloid extraction. Six alkaloids were isolated of which four were known, (+)-eburnamonine (1), (–)-eburnamine (2), (+)-isoeburnamine (3), (–)-kopsinine (4) [12–14], and two new ones, (–)-eburnaminol (5a) and (+)-larutensine (6). Structural elucidation was done by spectroscopic methods.

Eburnaminol (5a) was isolated in an amorphous form, $[\alpha]_D -54^\circ$ (CHCl₃; c 0.17). The UV spectra showed maxima typical of an indole chromophore: λ_{\max} nm (log ϵ) 228 (3.26), 278 (2.73), 290 (2.62) and the IR spectrum showed a broad hydrogen bonded hydroxyl band near 3450 cm⁻¹. The EI mass spectrum had a [M]⁺ at m/z 312 (37%), and with the aid of the ¹³C NMR data, molecular formula of C₁₉H₂₄N₂O₂, was deduced. Other fragmentation peaks observed were m/z 311 (34%, [M–1]⁺), 294 (20%, [M–H₂O]⁺), 281 (11%, [M–CH₂OH]⁺), 267 (29%, [M–C₂H₅O]⁺), 250 (100%, [M–C₂H₅O–OH]⁺) and 249 (98%, [M–C₂H₅O–H₂O]⁺). These peaks gave rise to the idea that there are two hydroxyl groups, of which one is in a CH₂CH₂OH chain. The ¹H and ¹³C NMR spectra were very similar to those of 2 (Table 1) which brought us to the conclusion that eburnaminol has an eburnane skeleton. In the ¹³C NMR

spectrum, a marked difference in the chemical shift values was observed at δ 58.4 and 40.7, which corresponded to C-18 and C-19, respectively; these values confirmed the presence of CH₂CH₂OH. Furthermore, in the ¹H NMR spectrum, a multiplet was observed at δ 3.80 due to the resonance of the C-18 hydrogens instead of a methyl triplet as in the case of 2. A singlet which most probably belonged to the isolated C-21 hydrogen was also apparent at δ 4.0. A *dd* centred at δ 5.61 (*J'* = 5 Hz, *J''* = 10 Hz) was assigned to H-16 which is vicinal to an hydroxyl group. These coupling constant values indicated that the hydrogen is situated in the axial position as in (–)-eburnamine (2). Because 5a is biogenetically related to all the known eburnane alkaloids isolated in this study, and possesses the same optical rotatory dispersion sign as that of (–)-eburnamine, we propose that (–)-eburnaminol (5a) has the same absolute and relative stereochemistry as all the known eburnane alkaloids mentioned above [15].

Larutensine (6) was also obtained in an amorphous state, $[\alpha]_D +22^\circ$ (CHCl₃; c 0.17). It exhibited a series of maxima typical of an indole chromophore in its UV spectrum: λ_{\max} nm (log ϵ) 226 (4.13), 278 (3.46) and 290 (3.45). The EI mass spectrum showed a [M]⁺ peak at m/z 294, and with the aid of ¹³C NMR data, the molecular formula was deduced to be C₁₉H₂₂N₂O. The fact that the molecular formula of 6 is 18 mu less than that of 5a suggests that it is formed by the loss of a water molecule from 5a, or its stereoisomer 5b. This hypothesis was supported from the IR spectrum which showed an absorption typical of a C–O stretching of an ether at 1080 cm⁻¹. Furthermore, the ¹³C spectrum revealed two peaks characteristic of two ether carbons at δ 77.5 and δ 58.5 which belong to C-16 and C-18 (confirmed by ¹H-¹³C COSY). From manipulation of the Dreiding molecular model, the formation of the ether by cyclization between C-18 and C-16 is only possible if the C-16 hydroxyl lies on the same side as the C-18 hydroxyl. Therefore, we concluded that 6 is derived from 5b. However, the hypothesized 5b has not been isolated in this particular work which may be due to its insignificant quantity, caused by its transformation to 6.

In addition, the ¹H NMR exhibited a *dd* at δ 5.8 (*J'*